



**Phase I Study Protocol of Autologous Multi-lineage Potential Cells (AMPC) on Acute Myeloid Leukaemia**

**Date:** 22 January 2019

**Version: 4.1**

## Acute Myeloid Leukemia Treatment with AMPC Private Trial Scope

<b>Primary Outcome Measures</b>	<p>To determine overall response rate (ORR) of peripheral blood-derived Autologous Multi-lineage Potential Cells (AMPC) in refractory or relapsed acute myeloid leukemia (AML) at 12 months</p> <p>Overall response rate (ORR) is defined as whether the patient achieves complete remission (CR) or complete remission with incomplete blood count recovery (CRi)</p> <p>CR Requirements:</p> <ul style="list-style-type: none"> <li>• Bone marrow aspiration shows less than 5% of abnormal blasts as determined by evidence from flow cytometry or immunohistochemistry</li> <li>• Bone marrow biopsy shows no clusters of blast cell</li> <li>• Normal values for absolute neutrophil count in peripheral blood exceeds 1,000/microL</li> <li>• Platelet count in peripheral blood exceeds 100,000/microL</li> <li>• Absence of extramedullary AML</li> </ul> <p>CRi Requirements:</p> <ul style="list-style-type: none"> <li>• All parameters of CR except platelet recovery or neutrophil recovery</li> <li>• Incomplete recovery—platelet count is less than 100,000/microL or neutrophil count less than 1,000/microL in peripheral blood</li> </ul>
<b>Secondary Outcome Measures</b>	<p>1) To determine safety profile and treatment-related adverse events (AE) upto 12-month follow up period</p> <p>AE is defined as any unintended or undesirable experience that occur during the course of the clinical investigation regardless of whether they are considered to be drug-related</p> <p>2) ORR at 3 and 6 months</p> <p>3) Overall survival (OS) rate at 12 months</p> <p>4) Time-to-next treatment (TTNT), defined as the time from the start of AMPC therapy to the start date of a subsequent line of therapy.</p>
<b>Title</b>	<p>The effects of AMPC in the treatments of refractory or relapsed AML</p>

<b>Brief Summary</b>	A private trial for evaluating the overall response rate contributed by AMPC in AML in refractory or relapsed AML
<b>Detailed Description</b>	<p>After inclusion and exclusion criteria has been determined and approved, written informed consent will be obtained from the candidate. All medical history relevant to the diagnosis of AML will be collected.</p> <p><u>Screening period:</u></p> <p>The screening period could extend from 0 to 7 days depending on the completion of screening laboratory results as below.</p> <p>On day -5 (up to day -1), the patient will undergo a screening test for the following test items:</p> <ul style="list-style-type: none"> <li>• BUN, creatinine, electrolyte, liver function test (LFT)</li> <li>• Full blood count (FBC), including blood smear</li> <li>• Hepatitis B/C</li> <li>• Human T lymphocytic virus type I and II (HTLV-I/II)</li> <li>• HIV1/2</li> <li>• Syphilis serology</li> <li>• Mycoplasma serology</li> </ul> <p>For Hepatitis B/C, HTLV-I/II, HIV1/2, Syphilis serology, and Mycoplasma serology, these tests obtained up to 3 months prior to day -5 can be allowed for using as screening result.</p> <ul style="list-style-type: none"> <li>• Chest X-ray</li> <li>• Bone marrow study including aspiration with wright's stain, biopsy, flow cytometry, and chromosome study (Any molecular testing for AML is optional.).</li> </ul> <p>Bone marrow biopsy can be omitted if the prior study performed within 14 days before day-5 and the available materials (core biopsy and slides) and result can be obtained for pathological review. In this case, only bone marrow aspiration for Wright's stain, flow cytometry, and chromosome study will be performed.</p> <p>Bone marrow biopsy will be repeated if the previous result has been performed more than 14 days prior to day -5 and/or FBC at day -5 reveals peripheral blast count higher than 10% of total white blood cells.</p>

	<p>For chromosome study (cytogenetics), the previous result before the recent line of chemotherapy prior to enrolment can be used for the screening data.</p> <p>On day 0, peripheral blood will then be collected, ranging from 250mL to 400mL depending on candidate fitness. The blood is collected into a sterile blood bag and sealed. Subsequent processes will be conducted in the blood bag within a closed-system to minimize contamination risks. FBC will be collected in order to determine the disease status. After that, the investigator will consider to prescribe blood transfusion for the candidate.</p> <p>On day 0 to day 3, the collected blood will be sent to the laboratory for stem cell culture, and a sample of the collected blood will be sent to a third-party laboratory for contamination testing of the following parameters:</p> <ul style="list-style-type: none"> <li>• Bacterial endotoxin</li> <li>• Total viable aerobic count</li> <li>• Total viable count</li> <li>• Microbial growth</li> <li>• Mycoplasma real-time PCR test</li> </ul> <p>On day 4, the biotest results will be released and the safety profiles of the AMPC product must be completed and passed before the cultured stem cells may be released for treatment</p> <p>On day 5, candidates will receive an infusion of the cultured stem cells. Prior to the infusion, FBC and blood chemistry (BUN, Cr, electrolyte, LFT) will be collected and the treating doctor will first conduct an allergy skin test to determine suitability for reinfusion. The cultured stem cells are then reinfused intravenously into the candidate in a process that could take up to 2 hours</p>
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	<p>The candidate participation will take place on day 0 to day 1 or 2 (if blood transfusion is required) and day 5 (period adjusted for blood transfusion if required) for peripheral blood collection and stem cell reinfusion respectively; with 12 month follow up after treatment;</p> <ul style="list-style-type: none"> <li>• 3 days post-treatment follow-up: full blood count test and blood smear, BUN, Cr, electrolyte, LFT</li> <li>• 10 days post-treatment follow-up: full blood count test and blood smear, BUN, Cr, electrolyte, LFT</li> <li>• 1 month (+/-7 days) post-treatment follow-up: full blood count test and blood smear, BUN, Cr, electrolyte, LFT, bone marrow study</li> <li>• 3 month (+/-7 days) post-treatment follow-up: full blood count test and blood smear, BUN, Cr, electrolyte, LFT, bone marrow study</li> <li>• 6 month (+/-7 days) post-treatment follow-up: full blood count test with bone marrow study</li> <li>• 12 month (+/-7 days) post-treatment follow-up: full blood count test with bone marrow study</li> </ul>
<b>Study Type</b>	Interventional
<b>Study Design</b>	Single-arm, open label, prospective study
<b>Condition</b>	Relapsed/Refractory Acute Myeloid Leukemia
<b>Intervention</b>	<p>Multi-lineage potential cells which were induced to de-differentiate from somatic leukocytes. Cells are autologous with respect to the patient, and are prepared in a suspension and administered via intravenous infusion.</p> <p>An estimated average of <math>1 \times 10^8</math> (<math>0.5</math> to <math>5 \times 10^8</math>) cells/per suspension(275 to 450mL) will be infused into the patient via intravenous infusion on day 5. Cell counts depend on yield of initial leukocyte harvest on day 0.</p>
<b>Study Arms</b>	<p>Experimental: Pilot study</p> <p>Sample size: 3</p>

	<p>To determine the effects of AMPC AML by monitoring the ORR and to observe in the long-term any manifestation of AE</p> <p>Experimental: Phase II of private study, which can be started only after complete 3 months of AMPC therapy in the first 3 patients in the pilot study and the safety profiles has been approved by investigator (and sponsor).</p> <p>Sample size: 7</p> <p>To determine the effects of AMPC on AML by monitoring the ORR and to observe in the long-term any manifestation of AE</p>
<b>Estimated Enrolment</b>	10
<b>Eligibility Criteria</b>	<p>Inclusion Criteria:</p> <ul style="list-style-type: none"> <li>• Must be unequivocally diagnosed with AML according to WHO classification with accompanying bone marrow biopsy and blood panel results</li> <li>• Must have refractory AML, defined as disease unresponsive to initial treatment; or relapsed AML that re-occured after treatment with conventional high dose chemotherapy</li> <li>• Candidates who have no available match-sibling donor for bone marrow transplantation (BMT) or are not suitable for BMT due to any reason.</li> <li>• Must have had prior treatment with chemotherapy at least 30 days prior to day 0 of this study and have recovered from treatment-related toxicity of chemotherapeutic agents with the exception of persistent diseases</li> <li>• Age 20 to 60 years old</li> </ul> <p>Exclusion Criteria:</p> <ul style="list-style-type: none"> <li>• Candidates who received any investigational therapies 4 weeks prior to treatment with this protocol</li> <li>• Candidates who received radiotherapy within 4 weeks prior to the treatment of this protocol</li> <li>• Candidates who have not recovered from any AE caused by radiotherapy</li> </ul>

	<p>or any agents received 4 weeks earlier</p> <ul style="list-style-type: none"> <li>• Candidates who have had a prior allogeneic stem cell transplant</li> <li>• Known case of extramedullary myeloid tumor (myeloid sarcoma)</li> <li>• Pregnant or breastfeeding women</li> <li>• Hydroxyurea has been prescribed within 10 days prior to day-5</li> <li>• Candidates have any abnormal screening laboratory results as below; <ul style="list-style-type: none"> <li>- Hemoglobin &lt; 9 g/dL</li> <li>- Total white blood cells count &gt; 30,000/microL (without ongoing G-CSF therapy)</li> <li>- Platelet count &lt; 75,000/microL</li> <li>- Creatinine clearance &lt; 30 mL/min/1.73 m<sup>2</sup> (by Cockcroft and Gault formula)</li> <li>- ALT &gt; 5x upper normal limit</li> <li>- Bone marrow study at screening period show blast &gt; 40% of total nucleated cells or severe hypocellularity (defined as &lt; 25% of normal cellularity for corresponding age) with presence of cluster of blasts</li> </ul> </li> <li>• Candidates have active heart disease including recent or chronic heart failure, unstable angina, recent acute myocardial infarction, or significant arrhythmia within 6 months of recruitment.</li> <li>• Candidates have concurrent malignancies unless the candidates have been free of the disease for at least 5 years.</li> <li>• Candidates positive for HIV1/2, hepatitis B/C, HTLVII, and Syphilis</li> </ul>
<b>Gender</b>	Male and Female
<b>Statistical Analysis Plan</b>	<p>Statistical Analysis Overview</p> <ul style="list-style-type: none"> <li>• For this open-label study, baseline characteristics and clinical primary and secondary outcomes will be descriptive analysed in order to explore the efficacy and treatment safety of AMPC in relapse/refractory AML patients who are not suitable for standard therapy.</li> <li>• There will be no statistical comparison between results from this phase I study and those from historical study as upper limit of total number of treatment patient is very low (N=10). Future phase II study might be</li> </ul>

	<p>planned after the completion of phase I study in order to further explore this novel treatment role in AML.</p> <p>Type of Statistical Test: Other (single group)</p>
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### **Clinical Protocol**

The procedures of the study are divided into four main steps:

1. Patient recruitment
2. Peripheral blood collection
3. AMPC product culture and analysis
4. Autologous reinfusion of product

### **Patient recruitment**

During patient recruitment, the investigator will inform volunteer of the objectives and procedure of the study. In order for a volunteer to be recruited, patient participation must be voluntary and volunteer must provide written informed consent to the participation in the study. After consent has been given by the patients, the necessary screening laboratory tests must be arranged for each patient according to the time frame set in the study design. The test items include:

- BUN, creatinine, electrolyte, liver function test (LFT)
- Full blood count (FBC), including blood smear
- Hepatitis B/C
- Human T lymphocytic virus type I and II (HTLV-I/II)
- HIV1/2
- Syphilis serology
- Mycoplasma serology

For Hepatitis B/C, HTLV-I/II, HIV1/2, Syphilis serology, and Mycoplasma serology, these tests obtained up to 3 months prior to day -5 can be allowed for using as screening result.

Bone marrow study including aspiration with wright's stain, biopsy, flow cytometry, and chromosome study (Any molecular testing for AML is optional.).

Bone marrow biopsy can be omitted if the prior study performed within 14 days before day-5 and the available materials (core biopsy and slides) and result can be obtained for pathological review. In this case, only bone marrow aspiration for Wright's stain, flow cytometry, and chromosome study will be performed.

Bone marrow biopsy will be repeated if the previous result has been performed more than 14 days prior to day -5 and/or FBC at day -5 reveals peripheral blast count higher than 10% of total white blood cells.

For chromosome study (cytogenetics), the previous result before the recent line of chemotherapy prior to enrolment can be used for the screening data.

The results of all screening testings must be completed before the determination of the eligibility for treatment according to inclusion and exclusion criteria. A baseline measurement of the outcome measures of the study will also be determined during this step. The volunteers will then be appointed for the "day 0" visit in order to inform the eligibility for further study participation and peripheral blood collection.

### **Peripheral blood collection**

Approximately 1 hour prior to blood collection, a temperature-controlled container of between 4 to 12°C is prepared. A universal identification number (UIN) label prepared for each patient and affixed on a 250mL blood bag with 35mL anticoagulant. The UIN must also include the patient's name, sex, date of birth, and date of collection.

Once blood collection is complete, the blood bag will be weighed and the weight is recorded. The tubes will then be sealed with a tube sealer to ensure minimal contamination from external environments. The blood bag will then be placed in the temperature-controlled container while ensuring that the temperature of between 4 to 12°C is maintained during transport of the product. After blood collection, the volunteers who have symptomatic anemia may be admitted for blood transfusion session.

### **Culture of the investigational product**

All laboratory technicians must check the blood sample upon receipt to ensure it is the correct

sample viable for processing. A laboratory label with the corresponding UIN must also be affixed to the blood sample.

The blood sample will be centrifuged for white blood cells and cultured according to the Manufacturing and Quality Aspects of Autologous Multi-lineage Potential Cells provided by Autologous Stem Cell Technology Pty Ltd to culture the investigational product. During this time, a sample of the product will be retrieved under a biosafety cabinet to be sent for contamination testing to ensure safety of the product prior to release.

### **Product analysis**

The investigational product will also be analyzed prior to release of sample for reinfusion to ensure quality of the product. These include white blood cell composition analysis, contamination testing, survival rate analysis, and cell surface marker analysis.

**Contamination testing:** Testing will be conducted by third party laboratories for total viable aerobic count, microbial growth, and endotoxin. The test results must reflect the safety of the sample prior to sample release to ensure safe administration of the product.

**White blood cell composition:** The complete blood count (CBC) of the sample must also be analyzed to determine the composition of leukocytes within the sample. The results will be broken down into monocytes, lymphocytes, and neutrophils.

**Viability testing:** The viability of leukocytes within the sample will be analyzed via flow cytometry with 7-AAD staining and the Trucount Tube to determine the composition of 7-AAD negative cells. The sample must reflect more than 60% cell viability.

**Cell surface marker analysis:** The cell surface markers of the sample population will also be determined via flow cytometry. The CD45+ cells and CD34+ cells within the population will be calculated with the Trucount Tube.

These tests results must be ready prior to reinfusion. Any logistical arrangements must take this into consideration. The comprehensive specifications of the product, including the test methods

and optimal results for each parameter is documented in the Manufacturing and Quality Aspects of Autologous Multilineage Potential Cells document prepared by Autologous Stem Cell Technology Pty Ltd.

## AMPC Product reinfusion

After the contamination testing that reflects sample safety is received, the sample will be released for reinfusion. The sample will be placed in a temperature controlled container of between 4 to 12°C and affixed with the correct UIN.

The investigator will confirm that the patient details match the UIN affixed on the sample and will then provide a 1mL stem cell allergy test on the patient. If this testing result at 30 minutes shows no allergic reaction, stem cell reinfusion will be commenced.

The blood bag containing the sample will first be connected to a blood giving set with a Y pump that is also connected to 0.9% sodium chloride solution. A 20 or 22 gauge needle will then be intravenously inserted into the patient's vein and a test sample of 5mL 0.9% sodium chloride solution infused through the needle to ensure correct needle placement. The connecting tubes from the blood giving set will then be connected to the needle and 0.9% sodium chloride solution will be infused for 5 minutes before the sample is infused in its entirety. During reinfusion, the nurse will maintain records of the duration of reinfusion, any physical changes, body temperature, blood pressure, pulse oximetry, and heart rate.

## Table of events

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